Model for Aerobic Growth of *Shigella flexneri* Under Various Conditions of Temperature, pH, Sodium Chloride and Sodium Nitrite Concentrations

ABSTRACT

A modified factorial design was used to measure the effects and interactions of temperature (10 to 37°C), pH (5.5 to 7.5), sodium chloride (0.5 to 5.0%), and sodium nitrite (0 to 1000 ppm) on the aerobic growth kinetics of *Shigella flexneri* in brain heart infusion broth. A total of 592 cultures were analyzed, with growth curves being generated using the Gompertz equation. A quadratic model for growth of *S. flexneri* in terms of temperature, pH, sodium chloride, and sodium nitrite concentrations was obtained by response surface analysis. This model provides an estimate of bacterial growth in response to any combination of the variables studied within the specified ranges. Estimates obtained with the model compared favorably with growth of *S. flexneri* in milk.

Shigella has been steadily gaining recognition as a causative agent of foodborne disease (*5,15*). Recently, an analysis of data associated with 7458 outbreaks involving 237,545 cases of foodborne disease reported to the Centers for Disease Control has been presented by Bean and Griffin (*2*). Among 2841 outbreaks of known etiology involving 124,994 cases, bacterial infections were responsible for 1869 outbreaks (66%) involving 108,906 cases (87%). *Shigella* were identified in 104 outbreaks (5.6%) involving 14,399 cases (13.2%) among diseases caused by bacteria. It is assumed that the data analyzed by the Centers for Disease Control represent only a fraction of shigellosis cases occurring in the United States. Based on a number of estimates, Todd (*18*) proposed 163,000 as a median number of foodborne shigellosis cases per year in the United States with an associated cost estimate of 63 million dollars. Estimates as high as 440,000 cases per year have been suggested (*1*).

Although mortality rates associated with shigellosis are relatively low in the United States (0.1%) (*2,18*), they are considerably higher in developing countries, particularly for young children (*9,17*). Epidemiological data indicate that poor personal hygiene was most frequently reported as a contributing factor in foodborne shigellosis outbreaks (*2*). The infectious dose of *Shigella* is very low, probably 10 to 100 organisms (*10*). Thus, it is not surprising that foods which are consumed raw or are not reheated after preparation (salads, salads with potato, chicken, seafood) are most often implicated in shigellosis outbreaks (*10,15*).

A model to estimate the growth of *Shigella* in food would be useful to the food processor, distributor, as well as to the consumer, to facilitate selection of conditions inhibitory to the organism. To develop such a model, detailed information is needed on the growth characteristics of the organism under various conditions relevant to the food of interest. Microbial growth depends on the interaction of a variety of factors such as temperature, pH, atmosphere, sodium chloride, and sodium nitrite concentrations. Response surface regression analysis of growth kinetics data has been used to develop multiple factor models for the growth of pathogenic bacteria including *Salmonella* (*8*), *Listeria monocytogenes* (*3,4*), *Clostridium botulinum* (*7*) and *Aeromonas hydrophila* (*11*).

Reports indicate that *Shigella* spp. are capable of surviving for considerable lengths of time in a variety of foods under various conditions (*13,14,16*). However, little systematic information is available on the growth characteristics of *Shigella* spp. (*6*). As part of our objective to develop a model for growth of *Shigella* in food, we have studied the growth characteristics of *Shigella flexneri* in a microbiological medium under various conditions of temperature, sodium chloride level, sodium nitrite concentration, and pH under aerobic conditions (*19,20*). The objective of the current study was to use these and additional data to derive mathematical models in relation to the aforementioned parameters that could be used to estimate the growth of *S. flexneri* in foods.

Microorganism

Shigella flexneri 5348 (obtained from Dr. David W. Niesel, University of Texas Medical Branch, Galveston, TX) was used throughout the study. To prepare the inoculum, the organism was cultured for 24 h in brain heart infusion (BHI, Difco Laboratories, Detroit, MI) at 37°C, and the culture was diluted with sterile 0.1% peptone water.

Experimental design

Data used to develop the bacterial growth models were obtained using factorial design (*19*) to assess the effects of pH (7.5, 6.5, 5.5), sodium chloride (0.5, 2.0, 3.5, 5.0%), and temperature (37, 28, 19, 10°C) (Experiment 1) and a partial factorial design (*20*) to assess the effect of sodium nitrite (0, 100, 200, 1000 ppm) in combination with pH (7.5, 6.5, 5.5), sodium chloride (0.5, 2.5, 4.0%), and temperature (37, 28, 19°C) (Experiment 2). Selected variable combinations were also tested at 12 and 15°C. All variable combinations were replicated at least three times.

Culture techniques

In Experiment 1, BHI broth was supplemented with 0, 15, 30, or 45 g/L sodium chloride to provide total concentrations of 0.5, 2.0, 3.5, or 5.0% sodium chloride, respectively. The pH was adjusted to 7.5, 6.5, or 5.5 using 1 N NaOH or 1 N HCl. The medium was dispensed in 100-ml portions into 500-ml Erlenmeyer flasks. The flasks were capped with foam plugs and sterilized by autoclaving for 15 min at 15 psi at 121°C.

In Experiment 2, BHI, diluted to 90% of final volume, was supplemented with 0, 20, or 35 g sodium chloride/900 ml; pH was adjusted to 7.5, 6.5, or 5.5 using 1 N NaOH or 1 N HCl. The media were then dispensed in 90-ml portions into 500-ml Erlenmeyer flasks and sterilized as above. Filter-sterilized sodium nitrite solutions (0, 1000, 2000, or 10,000 ppm) were added in 10-ml volumes to the sterile media. The final media contained 0.5, 2.5, or 4.0% sodium chloride and 0, 100, 200, or 1000 ppm (μg/ml) sodium nitrite.

All flasks were inoculated with 1 ml of a diluted 24-h culture of *S. flexneri* to an initial level of approximately 1 x 10^3 CFU/ml. All flasks were then incubated on a rotary shaker (150 rpm) at the desired temperature.

At appropriate intervals, samples were withdrawn from each flask by means of a pipet and the microbial population determined by surface plating on tryptic soy agar (Difco) using a Spiral Plater (Spiral System Instruments, Inc., Bethesda, MD). The plates (in duplicate) were incubated for 24 h at 37°C and counted.

Curve fitting

Growth curves were generated from the experimental data using the Gompertz equation (Table 1) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W. C. Damert (U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA), and copies are available upon request. The Gompertz parameter values (A, B, C, M) were subsequently used to calculate lag times (h), exponential growth rates [($\log_{10}$ CFU/ml)/h], generation times (h), and maximum population densities ($\log_{10}$ CFU/ml) as described by Gibson et al. (*7*) (Table 1).

Statistical analysis

Second order response surface models in terms of temperature, pH, sodium chloride concentration, and sodium nitrite concentration were calculated on the B and M Gompertz parameters for the *S. flexneri* growth data using least squares analysis of PROC GLM of the SAS System (*12*). The regression analysis was performed on the untransformed parameters B and M and on several transformations of the parameters including Ln(M) and Ln(B) (excluding no growth data), Sqrt(B), and Sqrt(1/M) (all data), Ln(B + 0.0001) and Ln[(1/M) + 0.0001] (all data).

TABLE 1. *Gompertz equation and associated equations for growth kinetics values.*

$$L(t) = A + C \exp [-\exp (-B (t-M))]$$

L(t) = Log count of the number of bacteria at time t (h).
A = Asymptotic log count as t decreases indefinitely (i.e., initial level of bacteria).
C = Asymptotic amount of growth (log number) that occurs as t increases indefinitely (i.e., number of log cycles of growth).
M = Time (h) at which the absolute growth rate is maximal.
B = Relative growth rate at M.

Associated Equations:

EGR = Exponential growth rate [($\log_{10}$ CFU/ml)/h] = BC/e
LPD = Lag phase duration (h) = M - (1/B)
GT = Generation time (h) = ($\log_{10}$ 2e)/BC
MPD = Maximum population density ($\log_{10}$ CFU/ml) = A + C

RESULTS AND DISCUSSION

Data from 592 cultures, representing 143 variable combinations, were used to derive the models to predict the aerobic growth of *S. flexneri* as a function of temperature, sodium chloride and sodium nitrite concentrations, and initial pH. Growth curves were generated using the Gompertz function (Table 1). This function has been used extensively in our laboratory to describe the growth of a variety of bacteria. Recently, Zwietering et al. (*21*) compared several sigmoidal functions to describe the bacterial growth curve and concluded that the Gompertz equation was statistically sufficient to describe the growth data of *Lactobacillus plantarum*, as well as other bacteria, and was easy to use. The growth kinetics data are not presented due to space limitations but are available on request. These data, summarized in our previous reports (*19,20*), indicate that the variables studied interact to affect the growth of *S. flexneri*.

The data base used to derive the mathematical models for aerobic growth of *S. flexneri* was obtained from the analysis of cultures with an initial level of approximately 1 x 10^3 organisms per ml. Additional experiments were carried out to determine the effect of inoculum level on growth kinetics. The data indicate that the growth kinetics are not substantially affected by the size of the initial inoculum. Table 2 shows the growth kinetics data for cultures at four inoculum levels of *S. flexneri* using the experimental parameter combination of 28°C, pH 5.5, 2.5% sodium chloride, and 0 ppm sodium nitrite. The inoculum level (Gompertz A value) affected the Gompertz C, B, and M values. However, regression analysis indicated that these terms varied in such a manner that the inoculum size had no significant effect on the derived values for exponential growth rate, generation time, and lag phase duration. For the vast majority of variable combinations, if the organism initiated growth, it achieved a maximum population density

TABLE 2. *Effect of inoculum size on growth kinetics*[a] *of* S. flexneri *in BHI (pH 5.5, 2.5% NaCl, 0 ppm $NaNO_2$) at 28°C.*

	A	C	B	M	EGR	GT	LPD	MPD
Mean	2.08	7.16	0.1180	15.17	0.311	0.97	6.70	9.22
Std.	(0.04)	(0.07)	(0.0013)	(0.32)	(0.003)	(0.01)	(0.25)	(0.06)
(n=3)								
Mean	2.85	6.43	0.1409	11.95	0.333	0.90	4.84	9.28
Std.	(0.03)	(0.07)	(0.0048)	(0.21)	(0.009)	(0.02)	(0.12)	(0.09)
(n=3)								
Mean	4.02	5.12	0.2198	11.51	0.414	0.73	6.95	9.14
Std.	(0.04)	(0.05)	(0.0107)	(0.14)	(0.019)	(0.04)	(0.34)	(0.04)
(n = 3)								
Mean	4.91	4.13	0.2005	9.72	0.304	0.97	4.87	9.04
Std.	(0.09)	(0.10)	(0.0084)	(0.51)	(0.006)	(0.02)	(0.40)	(0.04)
(n=3)								

[a] Abbreviations as in Table 1; std. = standard deviation, n = number of replicate cultures.

(A + C) of approximately 10^9 CFU/ml. On this basis, the Gompertz A and C values were omitted from consideration for model development. Similar observations and assumptions were reported by Gibson et al. (*8*), Buchanan and Phillips (*3*), and Palumbo et al. (*11*) in conjunction with the development of models for *Salmonella*, *L. monocytogenes,* and *A. hydrophila*, respectively.

The growth data were subjected to second order response surface analysis of the Gompertz B and M values as functions of temperature, pH, sodium chloride concentration, and sodium nitrite concentration. Four models were obtained in the form of quadratic polynomial equations shown in Table 3. In Model 1, regression analysis was performed on B and M values without transformation. In cases where growth did not occur (B = 0), M values were eliminated since these were equal to infinity. Natural logarithm transformations have been found to be effective in the development of bacterial growth models (*3,8*). In Model 2, natural logarithm transformations, Ln(B) and Ln(M), were used with elimination of data where growth did not occur (B = 0). To obtain Model 3, the no-growth data were included using Ln[(1/M) + 0.0001] and Ln(B + 0.0001) transformations; the constant value of 0.0001 was added to avoid B or 1/M values of zero that could not be analyzed by a Ln function. Regression analysis was also performed using the square root transformation, Sqrt(B), and Sqrt(1/M), in order to include all data (Model 4).

Examination of the R^2 values for the four sets of equations (Table 3) indicated that Model 2 would be expected to provide the best fit. Direct comparison of generation time and lag phase duration values calculated using Models 2, 3, and 4 with corresponding experimentally observed values for a number of variable combinations are given in Table 4. Gompertz parameters A = 2.93 and C = 6.20, the grand means of the experimental data, were used in conjunction with the values obtained for B and M to obtain the growth kinetics data. We conclude that the best overall fit is obtained with Model 2. Evaluation of Model 1 (data not shown) indicated that it gave a considerably poorer fit between calculated and experimentally observed values than models with logarithmic transformations. The F values obtained for Model 2 are shown in Table 5. Most variables and the intercept are highly significant ($P<0.001$).

To test the effectiveness of the selected model (Model 2), we studied the growth of *S. flexneri* in milk at 28 and 19°C. Table 6 shows the calculated values of exponential growth rates, generation times, lag times and maximum population densities, and the corresponding experimentally observed values for milk as well as BHI broth having similar pH (6.5) and sodium chloride content (0.5%). The results suggest that Model 2 provides a reasonable estimate of *S. flexneri* growth in this dairy food. It should be pointed out that the larger value for lag phase duration with a large standard deviation for the BHI cultures grown at 19°C is due to the fact that three of the nine cultures were unusually slow growing. If data for the three slow-growing cultures are excluded, the remaining six BHI cultures at 19°C yield the following values and standard deviations: exponential growth rate = 0.087 (0.005), generation time = 3.5 (0.2), lag phase duration = 10.6 (3.6), maximum population density = 9.9 (0.2). Since data from all nine cultures were used in model development, a certain bias toward slower growth under these conditions may exist. Regretfully, there are virtually no published data on the growth kinetics of *Shigella* that we could use to evaluate the effectiveness of the model in relation to other food systems.

Additional comparisons based on a variety of foods and growth conditions need to be carried out in order to obtain a fuller evaluation of the growth model. Undoubtedly, there are additional parameters that influence the growth of *S. flexneri* that may play an important role in particular types of food. These may need to be taken into consideration in order to refine the growth model. However, we consider that the model as presented here (Model 2) can be useful to persons involved in the manufacture, storage, or handling of food to estimate the likelihood that

TABLE 3. *Quadratic models for the effects and interactions of temperature (T) (°C), initial pH (P), sodium chloride concentration (S) (%), and sodium nitrite concentration (N) (ppm) on the aerobic growth of* S. flexneri.

Model 1

$B = -1.348 - 0.01134T + 0.3918P + 0.113S - 0.00063N + 0.00271TP - 0.00215TS - 0.00000316TN - 0.01056PS + 0.000049PN + 0.0000247SN + 0.000171T^2 - 0.0309P^2 - 0.00282S^2 + 0.000000264N^2.$
($R^2 = 0.750$, adjusted $R^2 = 0.819$[a], n = 592)

$M = 1886.4 - 67.9T - 241.893P - 5.044S + 1.1585N + 2.401TP - 1.176TS - 0.0132TN + 5.0469PS - 0.088874PN + 0.01237SN + 0.84477T^2 + 11.281P^2 + 3.329S^2 - 0.000103N^2.$
($R^2 = 0.644$, adjusted $R^2 = 0.810$, n = 373)

Model 2[b]

$Ln(B) = -20.011 + 0.5746T + 2.256P + 0.2027S - 0.00785N - 0.007273TP - 0.000646TS + 0.000035TN - 0.0297PS + 0.000764PN + 0.000203SN - 0.00699T^2 - 0.13298P^2 - 0.046804S^2 + 0.0000006N^2.$
($R^2 = 0.870$, adjusted $R^2 = 0.920$, n = 373)

$Ln(M) = 36.839 - 0.7082T - 6.607P - 0.5845S + 0.0201N + 0.01235TP - 0.00545TS - 0.000082TN + 0.0974PS - 0.00225PN + 0.0000335SN + 0.00857T^2 + 0.45034P^2 + 0.08559S^2 - 0.0000009N^2.$
($R^2 = 0.895$, adjusted $R^2 = 0.926$, n = 373)

Model 3

$Ln(B+0.0001) = -82.455 + 0.898T + 18.297P + 1.912S - 0.0383N - 0.00566TP - 0.00860TS + 0.0000959TN - 0.238PS + 0.00331PN - 0.000174SN - 0.0113T^2 - 1.249P^2 - 0.1486S^2 + 0.0000111N^2.$
($R^2 = 0.654$, adjusted $R^2 = 0.732$, n = 592)

$Ln[(1/M)+0.0001] = -81.577 + 0.778T + 18.48P + 2.09S - 0.0344N - 0.00225TP - 0.0118TS + 0.0000667TN - 0.2572PS + 0.00287PN - 0.000092SN - 0.0094T^2 - 1.281P^2 - 0.1373S^2 + 0.0000108N^2.$
($R^2 = 0.685$, adjusted $R^2 = 0.750$, n = 592)

Model 4

$Sqrt(B) = -3.397 + 0.0127T + 0.904P + 0.172S - 0.00182N + 0.0030TP - 0.00255TS + 0.00000008TN - 0.01695PS + 0.000152PN + 0.00002SN - 0.000146T^2 - 0.0668P^2 - 0.007749S^2 + 0.00000059N^2.$
($R^2 = 0.755$, adjusted $R^2 = 0.813$, n = 592)

$Sqrt(1/M) = 3.2095 + 0.0108T + 0.8757P + 0.153S - 0.00129N + 0.00178TP - 0.00214TS - 0.0000026TN - 0.01547PS + 0.000104PN + 0.0000195SN - 0.000053T^2 - 0.06455P^2 - 0.006465S^2 + 0.00000048N^2.$
($R^2 = 0.780$, adjusted $R^2 = 0.812$, n= 592)

[a] Adjusted $R^2 = R^2/\text{Max. } R^2$. (N. R. Draper and H. Smith. 1981. Applied regression analysis, 2nd ed. John Wiley & Sons, Inc. pp. 40-42).
[b] No growth responses were treated as missing values.

Shigella, if present, would grow under a given set of conditions. A computerized program has been developed in our laboratory that makes available, in a user-friendly manner, this growth model for *S. flexneri*.

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TABLE 4. *Comparison of selected examples of experimentally observed growth kinetics values for* S. flexneri *vs. values obtained with Models 2, 3, and 4.*

				Model 2		Model 3		Model 4		Observed	
T	S	P	N[a]	GT	LPD	GT	LPD	GT	LPD	GT	LPD
10	0.5	5.5	0	141.3	4070.5	NG[b]	NG	6.3	16.4	NG	NG
10	2.0	7.5	0	81.9	2606.6	1459.7	13840.3	138.7	-1185.2	NG	NG
12	0.5	6.5	0	37.8	470.1	289.8	680.0	115.3	639.1	NG	NG
15	0.5	6.5	0	13.7	124.3	47.0	164.2	10.1	61.2	15.0	180.1
19	0.5	6.5	0	4.3	26.0	6.9	34.9	2.8	16.5	4.7	33.0
19	0.5	6.5	200	6.4	75.3	95.7	423.6	21.8	206.5	13.0	168.0
19	2.5	6.5	200	7.9	128.4	175.5	645.3	29.6	277.8	NG	NG
19	4.0	6.5	100	10.4	197.8	167.9	502.1	23.5	233.5	NG	NG
19	0.5	7.5	0	3.4	31.8	3.8	31.2	2.4	20.7	2.3	44.2
19	0.5	7.5	1000	7.2	67.3	7.3	93.0	5.2	80.0	8.8	80.0
19	2.5	7.5	200	5.6	100.1	75.7	635.7	20.0	833.9	5.0	130.9
19	4.0	7.5	100	8.4	240.8	152.8	1235.4	57.2	123733.4	NG	NG
28	0.5	5.5	0	1.1	8.4	7.6	37.1	2.0	12.5	1.1	3.5
28	0.5	5.5	100	1.4	17.9	40.3	170.2	4.8	36.9	1.1	59.3
28	2.5	5.5	0	1.4	8.0	9.0	40.3	2.6	16.0	1.2	15.3
28	2.5	5.5	100	1.8	19.1	49.3	182.6	6.9	51.7	NG	NG
28	4.0	5.5	0	2.2	15.6	22.3	80.1	5.1	35.3	2.5	19.3
28	0.5	6.5	0	0.7	2.0	0.4	2.4	0.7	3.7	0.7	2.9
28	2.5	6.5	100	1.1	5.5	2.7	16.1	1.5	9.3	1.4	4.8
28	4.0	6.5	100	1.7	14.6	9.6	50.7	3.0	22.6	1.3	13.0
28	5.0	6.5	0	2.4	21.0	8.2	41.6	3.3	26.8	2.4	9.5
28	0.5	7.5	0	0.6	3.6	0.2	2.0	0.6	4.0	0.6	3.9
28	0.5	7.5	1000	0.9	1.2	0.2	3.6	0.8	9.4	0.7	3.9
28	2.5	7.5	0	0.9	7.0	0.7	6.6	0.9	7.7	1.6	6.3
28	2.5	7.5	100	0.9	7.9	1.8	18.6	1.3	12.5	1.5	7.1
28	4.0	7.5	0	1.5	20.0	3.3	32.3	1.9	22.9	1.2	45.6
37	0.5	5.5	100	0.7	7.1	11.4	29.2	1.2	5.8	0.8	2.8
37	2.5	5.5	0	0.7	2.5	3.3	10.6	1.1	5.0	0.6	1.8
37	4.0	5.5	0	1.1	4.2	9.2	26.0	2.2	10.2	0.8	4.9
37	4.0	5.5	1000	2.8	587.5	NG	NG	35.7	-5.3	NG	NG
37	0.5	6.5	0	0.4	1.0	0.1	0.5	0.3	1.6	0.3	2.2
37	0.5	6.5	200	0.5	2.3	1.1	4.7	0.6	3.0	0.5	2.1
37	2.5	6.5	100	0.6	1.7	0.9	4.4	0.7	3.6	0.7	0.8
37	4.0	6.5	0	0.8	2.4	1.1	4.9	0.9	4.7	0.8	3.1
37	4.0	6.5	200	0.9	6.8	11.1	46.9	1.9	13.2	0.7	5.3
37	0.5	7.5	0	0.3	2.2	0.1	0.4	0.3	1.6	0.4	2.1
37	3.5	7.5	0	0.7	6.0	0.8	6.1	0.6	5.1	0.6	6.9
37	4.0	7.5	100	0.8	9.1	3.9	31.7	1.1	11.5	0.7	4.1
37	5.0	7.5	0	1.3	20.6	7.0	52.6	1.6	21.1	2.8	0.2

[a] Abbreviations as in Tables 1 and 3. [b] NG = no growth.

TABLE 5. *F-Values for variables[a] for Model 2.*

	Ln(B)		Ln(M)	
Variable	F Value	(P > F)[b]	F Value	(P > F)
Intercept	63.20	0.0001	183.87	0.0001
T	138.08	0.0001	180.28	0.0001
P	12.00	0.0006	88.44	0.0001
S	1.70	0.1927	12.17	0.0005
N	22.94	0.0001	129.78	0.0001
TP	2.22	0.1372	5.50	0.0196
TS	0.09	0.7690	5.28	0.0221
TN	7.37	0.0069	34.54	0.0001
PS	2.38	0.1241	21.95	0.0001
PN	13.42	0.0003	99.51	0.0001
SN	10.73	0.0012	0.25	0.6168
T^2	180.26	0.0001	232.52	0.0001
p^2	8.73	0.0033	86.04	0.0001
S^2	19.42	0.0001	55.82	0.0001
N^2	3.32	0.0691	6.80	0.0095

[a]Abbreviations as in Table 3. [b]Probability of a larger value of F.

TABLE 6. *Growth kinetics for* S. flexneri *observed in milk and in BHI at 28 and 19°C and estimated by Model 2.*

	Exponential growth rate, (log_{10} CFU/ml)/h	Generation time, h	Lag phase duration, h	Maximum pop. density, log_{10} CFU/ml
28°C				
Milk[a] (n = 3)	0.389 (0.017)[c]	0.8 (0.1)	1.9 (0.2)	9.0(0.1)
BHI[b] (n= 11)	0.477 (0.028)	0.6 (0.1)	2.9 (0.3)	9.9 (0.2)
Estimated	0.415	0.7	2.0	9.1
19°C				
Milk (n = 3)	0.071 (0.006)	4.2 (0.3)	11.1 (2.6)	9.0 (0.1)
BHI (n = 9)	0.073 (0.022)	4.6 (1.8)	29.0 (28.5)	9.9 (0.1)
Estimated	0.070	4.3	26.0	9.1

[a]Whole milk, UHT, Grade A, Vit. D, pH 6.5, approx. 0.5% NaCI, inoculated with *S. flexneri* to give 7.4 x 10^2 CFU/ml. Incubation and determination of bacterial population were carried out as described for BHI cultures (Materials and Methods).

[b]Brain Heart Infusion broth, pH 6.5, 0.5% NaCI, inoculated with *S. flexneri* to give approx. 1 x 10^3 CFU/ml. [c]Standard deviation.